Exhibit 3

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POLYCLONAL AND MONOCLONAL ANTIBODIES

Polyclonal Antibody

Immunization of Mice:

Injections should be made at intervals of at least two weeks.. We have been using either of the two following adjuvants with success: Freund adjuvant and MPL+TDM adjuvant (RIBI ImmunoChem Research, Inc.), the latter being less hazardous than the first.

1) 1st injection: 15 - 50 ug Antigen in 200 ul adjuvant, injected subcutaneously.

2) 2nd injection: 2 weeks later; boost with 15 - 50 ug of Antigen in adjuvant. (If time permits, boost again a month later).

3) 7 - 10 days later bleed the mouse and test serum using the assay which will be used for screening. If titre is not high enough, boost again two weeks after previous boost.

Serum Preparation:

1) After collecting, blood should be allowed to clot for 60 min . at 37¡C or O.N at 4¡C.

2) Separate the clot from the sides of the tube (ringing) using a pasteur pipette. Place clot at 4¡C O.N.

3) Spin at 10000 x g for 10 min. at 4¡C to separate the serum.

4) Serum can be stored at -20¡C after adding Glycerol to 50%.

If monoclonal antibodies are desired, once you have a "good" polyclonal and a reliable screening method proceed to the next step.

Rabbits.

Rabbits are immunized essentially as described above. Injections:

1. First in Freund's complete 100-200ug/1ml injection, two sites (over each shoulder).

2. Second 1 month later in Freund's INCOMPLETE as above.

3. Bleed 2 weeks later. Boost 2 weeks later again (2 + 2 week cycle).

Monoclonal Antibody

If titre is positive do one of the following 2 weeks later:

I - Boost tail vein with 20 ug- 50 ug of Antigen in PBS and proceed with fusion on day 4.

OR

II - Boost subcutaneously with 50 ug - 100 ug of Antigen in PBS and proceed with fusion 4 days later.

OR

III - Boost 3 days in a row with 15 ug Ag in PBS and proceed with fusion on 4th day.

Cell Lines:

1) Myeloma; P3X63-Ag8.653

Origin: BALB/c, non secreting, 8-azaguanine resistant, HPRT -.

2) Myeloma fox-NY

Origin: Robertsonian, 8-azaguanine resistant, HPRT -, APRT -.

(mice have resistance to drug and expression of heavy chain on the same chromosome).

3) Macrophage-derived J774A.1

Maintenance of the cells:

Stock solutions:

¥ IMDM:

¥ Fetal Calf Serum-

¥ Transferrin: Iron saturated. 1000X stock = 1 mg/ml

¥ HT supps: 50X from Sigma H0137 (Store at -20¡ C).

¥ 2-Mercaptoethanol: 1000X stock (5 x 10 -2). (Store at 4¡C.)

¥ AT supplement : 50X stock , Sigma A-7422. (Store at -20¡C.)

¥ Kanamycin Sulfate: 100X from Gibco-BRL 600-5160AG . (Store aliquots at -20¡ C)

¥ MCM: Macrophage Conditioned Medium. (used instead of feeder cells)

Seed macrophages at a density of 1.5x105 cells/ml in the medium described on next page. Add 2.5 ug/ml LPS which induces differentiation.

Collect sup after 2-3 days, or when medium is getting too yellow. Induce 2 more times , each time with 1 ug/ml LPS and collect sup after 2 days each.

Pool the sups, filter and use as recomended. (Could be aliquoted and stored at -20C.)

Preparation of Media:

for P3X63-Ag8.653: IMDM complete

to 425 ml of IMDM add:

0.5 ml 1000X transferrin

0.5 ml 1000X 2- Mercaptoethanol

10 ml 50X HT

5 ml 100X Kanamycin Sulfate

75 ml FCS (final 15%)

for fox-NY: IMDM or RPMI

10 % FCS

1X AT supplement

transferrin

Kanamycin

for J774A.1: same medium as the one for the P3X63-Ag8.653 (= Ag8).

Growth conditions:

All cell lines mentioned above grow at 37¡C, 7% CO2 .

The Myelomas optimal density is 3.5x105/ml.

The Macrophages optimal density is 1.5x105/ml. When expanding them , use a "policeman" to scrape them; this is easier to do if they are growing in petri dishes at this stage.

Freezing Hybridoma / Myeloma / macrophages.

Freezing solution: 90% FCS + 10% DMSO, ice cold.

1) Spin down 107 cells (106 minimum) at 1200 rpm for 5 min.

2) Aspirate medium.

3) Resuspend in 1 ml of ice cold freezing solution.

4) Transfer vial to an insulated freezing box and place at -70¡C for at least 1 hr. (could be for a couple of days).

5) Transfer the vial from the -70¡C to the liquid nitrogen tank and log the entry in the freezer log.

Thawing cells:

1) Take vial out of liquid nitrogen tank and thaw it immediately in a 37¡C bath (about 1 min).

2) When there is still a small piece of ice left, dilute the cells by transfering them into a conical tube containing 10 ml of the growth medium at 37¡C.

3) Spin at 1200 rpm for 5 min.

4) Aspirate medium and resuspend cells in 5 ml of medium, in a 25cm2 flask.

Cell Fusion and Selection

Solutions:

¥ IMDM

¥ IMDM complete + 15% MCM.

¥ PBS

¥ PEG 50% (w/v): PEG 4000 (50%) from Boehringer Manheim 1 243 268

¥ Aminopterin: Sigma, A 5159 (50X)

1) Prepare a T-150 flask with 170ml of IMDM complete with MCM instead of PCM (= IMDM - m), and keep it in the incubator for fused cells.

2) Isolation of spleen cells :

a) Sacrifice mouse by cervical dislocation. Immerse mouse in 70% Ethanol.

b) Remove spleen (on the left side) and transfer into a small petri dish which contains IMDM at room temp. Clean fat from the spleen and transfer the spleen into an empty dish.

c) Using sharp tipped forceps, one end is punctured. A curved forceps is used to hold down the intact end, and the spleen is gently rubbed towards the opened end with another set of forceps. The cells from inside the spleen will ooze out with very little damage. Stop the process when you are left with a nearly empty, transparent skin. Collect the cells by rinsing with IMDM.

d) Transfer cell suspension to a 15ml conical tube and let the cell debris settle out (approx. 5 min.).

e) Remove the cell suspension (without disturbing the settled cell debris) and transfer to a 50ml conical tube. Add an additional 30ml of IMDM and pellet the cells at 1200 rpm for 5 - 10 min.

f) Aspirate the medium, resuspend pellet and wash again with 30ml of IMDM. One immunized spleen has aprox. 108 cells. After this wash the cells are ready for the fusion.

3) Myeloma cell preparation:

It is essential that the myelomas be free of debris, rounded and refractive under phase contrast, and that they are harvested in log or late log phase growth (between 3.5 and 9 x105 cells/ml).

a) Thaw cells 7 days before scheduled fusion. Myeloma do not grow well after being in culture for more than a few weeks.

b) Make sure you refreeze cells for future use.

c) We have been using a ratio of 2 spleen cells : 1 myeloma cell. However, workers have been using a ratio from 1 to 10 spleen cells per myeloma successfuly. For one spleen we harvest 5x107 cells. It is advisable to do this spin at the same time that the second wash of the spleen cells is done.

4) The fusion:

a) The washed myeloma and spleen cells are pooled in 30ml of PBS (room temp.) and spun gently at 1000 rpm for 10 min.

b) Aspirate the PBS and resuspend pellet gently by tapping the tube. Volume should be approx. 0.8 ml.

c) Set a timer.

d) Add an equal volume of PEG solution, slowly, dropwise, with gentle tapping, over 1.5 min. at room temp. Then gently wiggle the tube for 1.5 min.at 37¡C. Some cell clumping will be evident.

e) The suspension is spun at 1000 rpm for 3-4 min. (at this point you should see the different layers of cells with PEG on top).

f) Slowly add 37¡C IMDM to 10 ml, without disturbing pellet. After adding , swirl the tube gently to mix and dilute the PEG. Do not disturb the cells.

g) Spin at 1000 rpm for 5 min.

h) Aspirate medium, resuspend cells by tapping. Slowly add 5 ml of 37¡C IMDM-m.

i) Bring to 20 ml and add to the flask in the incubator. (If using feeder cells, add them at this point; 106 cells/ml).

j) Add Aminopterin (2ml to 200 ml of medium). (Some workers will leave the cells at this point for 24 hours before adding Aminopterin. We add the drug immediately.)

k) Seed the cells in 96 well microtiter dishes, 250 ul per well, 8 plates per fusion.

First clones may be seen in 7-10 days. First screen will usually start after 2 weeks, with a second and third, if necessary, a few days later.

Screening

ELISA

Materials:

¥ Plates - 96 well Dynatech Immulon, type 2. (Fisher 17-0221-199).

¥ PBS, TBS

¥ PBS + 0.1% Tween 20 or TBS + 0.1% Tween 20.

¥ Blocking solution = 2% BSA (type V) in PBS. (Add 0.02% azide for longer storage.)

¥ Elisa buffer = 2% BSA + 0.1% Tween 20 in PBS (azide optional).

¥ Enzyme linked antibody = Horseradish peroxidase 1

¥ Substrate = ABTS - 100X (from Zymed 00-2001)1

¥ HRP buffer: 100mM Na Citrate pH 4.2(490 mg citric acid + 720 mg Na citrate

dihydrate + 50 ml H2O, pH 4.2).

¥ Hydrogen peroxide 30% (1000X)

Protocol:

1) ADSORBTION OF ANTIGEN

a) Dilute Ag to 10 ug/ml in PBS.

b) Add 100 ul of Ag solution to each well.

c) Leave O.N covered with saran wrap, at 4¡C.

2) BLOCKING

a) Wash unbound Ag by inverting the plates and flicking the wells dry.

b) Rinse by adding PBS to each well and inverting it again (use squirt bottle).

c) Repeat the rinse twice.

d) Add 100 ul of blocking solution to every well, leave 1 hr at room Temp or O.N at 4¡C.

3) PRIMARY ANTIBODY

a) Add the antibody to be tested :

Sup of cells = 25 ul , mix well by pipeting up and down (10 times).

serum, ascites = 1:100 and a serie of 1/5 dilutions. Do dilutions in blocking solution.

b) Leave 1 hr at room temp or O.N at 4¡C.

4) SECONDARY ANTIBODY

a) Wash unbound antibody 4 times with PBS + 0.1% Tween 20.

b) Add 100 ul of enzyme linked antibody to all wells. Do the appropriate dilutions in the Elisa buffer. (ex: HRP is 2000X).

c) Leave 1 hr at room temp or O.N at 4¡C.

5) SUBSTRATE

a) Disolve substrate in water.

b) Wash plate 4 times with PBS + 0.1% Tween 20 (use TBS instead of PBS for AP).

c) Add 100 ul of substrate to every well.

d) Watch color development. This could take from a few seconds to 20 min.

e) If needed, stop the reaction by adding 50 ul of 4M NaOH.

f) Read absorption in Elisa reader at correct wavelength (for HRP system 416 nm, for AP - 405 nm).

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Last modified on 10/24/95.

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1 This is the system we have been using mostly, but there are other linked antibodies available which can be used with the same procedure.